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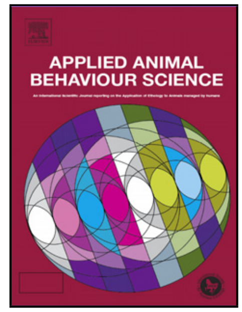

Title: Does positive reinforcement training affect the behaviour and welfare of zoo animals? The case of the ring-tailed lemur (*Lemur catta*)

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PII: S0168-1591(17)30228-9
DOI: http://dx.doi.org/doi:10.1016/j.applanim.2017.07.007
Reference: APPLAN 4496

To appear in: *APPLAN*

Received date: 14-2-2017
Revised date: 11-7-2017
Accepted date: 27-7-2017

Title: Does positive reinforcement training affect the behaviour and welfare of zoo animals? The case of the ring-tailed lemur (*Lemur catta*)

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Effect of PRT on ring-tailed lemur welfare

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Highlights

- Isolation training programmes might be stressful for social animals as lemurs.
- Positive Reinforcement Training (PRT) is widespread in modern zoos and laboratories.
- Research on the effect of PRT in primates outside the training sessions is needed.
- PRT seems to benefit ring-tailed lemur social intra- and inter-specific behaviour.
- Individual variability in the response of lemurs during the training was found.

Abstract: Positive reinforcement training (PRT) is an established tool to facilitate animal husbandry, care and research in modern zoos, with potential positive implications for captive animal welfare. The study explored the role of an isolation PRT training programme on the well-being of ring-tailed lemurs (*Lemur catta*). Eleven subjects were observed during an isolation training protocol to induce the animals to enter an area (training area) calmly and retrieve rewards separated from group members. Duration of individual and social behaviours were collected over two different periods: the baseline period, before the beginning of the isolation training protocol and the training period, in which the collection of the data started at the end of the isolation training sessions. Additionally, behavioural data within the isolation training sessions (latency to enter the training area and retrieve the reward, display of stress-related behaviours) were recorded. Outside the training sessions, lemurs were out of sight significantly more in the baseline (Mean ± SD: 15.46 ± 5.20) than in the training (Mean ± SD: 4.36 ± 2.89) period. Social behaviour was performed significantly more in the training (Mean ± SD: 31.80 ± 12.34) than in the baseline (Mean ± SD: 12.52 ± 5.14) period; particularly, lemurs were in social contact significantly more in the training (Mean ± SD: 14.09 ± 6.00) than in the baseline period (Mean ± SD: 4.58 ± 2.73). Agonistic behaviours were performed significantly more in the baseline (Mean ± SD: 0.23 ± 0.15) than in the training (Mean ± SD: 0.07 ± 0.07) period. Within the training sessions, all the individuals entered the training area, were isolated from conspecifics, and retrieved the reward in 6 out of 9 sessions. Our findings show that, during the PRT period, lemurs displayed their natural behaviour in their everyday social life with significant increase of their affiliative behaviours and decrease of aggressive behaviours with benefits for their welfare status. Thus, lemurs were able to cope with the use of PRT to isolate each individual from its social group – a situation which, without training, might be very stressful. In conclusion, PRT may play a crucial role for the captive management of ring-tailed lemurs in captive facilities, including zoos.

Keywords: *Lemur catta*, training programme, captive primate behaviour, animal well-being

Manuscript word count: 5,137

1. Introduction

Positive reinforcement training (PRT) based on operant conditioning learning is used widely in modern zoos and laboratories to train animals to perform specific behaviours voluntarily and cooperate with caretakers, researchers and vets (Skinner, 1938; Skinner, 1966). PRT is commonly used in biomedical research, producing reliable results and guaranteeing the well-being of animals in laboratory settings (Laule, Bloomsmith & Schapiro, 2003; Scott et al., 2003). In the last decades, this technique has become widespread and increasingly relevant among modern zoos, enhancing animal husbandry standards and welfare. Moreover, PRT in zoos provides the opportunity for non-invasive research on animals in semi-natural environments.

Rewarding the performance of an action increases the likelihood of that action happening again, causing an animal to comply with a requested behaviour - such as moving from one area to another. Obtaining the voluntary cooperation of animals in husbandry, veterinary, and research protocols might have positive implications on animal welfare. It allows a desensitization to stressful and frightening events (Laule et al., 2003; Reinhardt et al., 1990), an enhanced welfare by providing mental stimulation through challenging and learning opportunities (Laule & Desmond, 1991; van Praag et al., 2000), and improved human-animal interactions (Hosey & Melfi, 2012). Training programmes in zoos and other facilities may also involve other techniques, such as negative reinforcement training and punishment (reviewed in Prescott et al., 2005; Pryor, 1999). However, differently from other techniques, PRT, by using a positive reward, helps to establish a relaxed atmosphere in stressful contexts, giving animals the opportunity to choose and control the situation, with positive implications on their welfare (Lambeth et al., 2006; Laule, 2010; Laule et al., 2003;

Laule & Whittaker, 2007; Pryor, 1999; Reinhardt, 1992). Similarly to environmental enrichment, PRT provides animals with choices and control over the environment, promotes species-specific behaviour, and allows the animals to cope better with new stimuli and challenges (Westlund, 2014, 2015). These factors have been found to enhance psychological and physical well-being (Westlund, 2015). In addition, previous studies in chimpanzees (*Pan troglodytes*) reported that PRT programmes, such as retrieving rewards, might reduce inter-specific aggression and abnormal behaviour outside the training context (Baker, 2004).

Furthermore, investigating whether species-specific behaviours are exhibited by captive individuals and comparing the behavioural patterns of animals in captivity to those of wild conspecifics are considered valuable methods of assessment for the psychological and physiological well-being of captive animals (see Hill and Broom, 2009; Hosey et al., 2013). The presence of abnormal and stress-related behaviours, such as over-grooming, might also indicate poor welfare conditions and high stress levels of the individuals (Dawkins, 1990). These behaviours may be due to negative experiences, such as inability to perform species-specific behaviour or stressful husbandry procedures (Jacobson et al., 2016; Lutz et al., 2003). Stereotypies and self-injurious behaviours such as pacing, rocking back and forth, self-clasping and eye-poking (Bayne and Novak 1998; Birkett & Newton-Fisher 2011; Capitanio 1986; Erwin & Deni 1979) are examples of abnormal stress-related behaviour in non-human primates. Their occurrence might help the animal to reduce stress and deal with the presence of an inadequate environment or experience (Mason and Latham 2004). As well as this, depending on the amount of time, other behaviours may indicate the presence of stress in a new or unpredictable situation for the animals; for instance, locomotion, defecation, urination and over-grooming (Archer, 1973; Ramos & Mòrmede, 1998; Spiezio et al., 2015). In particular, in such situations, locomotion and defecation have been found to act as valid emotional measures (Lister, 1990; Ossenkopp et al., 1994). Regarding social behaviour in group-living primates, stressful situations have been previously related to an increase of aggressive behaviours (Novak & Suomi, 1988); as very social species, non-human primates are expected to display high levels of affiliative, exploratory and playful behaviour, together with low levels of aggression and stereotypic activities (Novak & Suomi, 1988).

Though training is widespread among modern zoos, few studies have investigated the impact of this procedure on animal welfare or the responses of individuals to the training condition - especially in strepsirrhines (see Melfi, 2013). In particular, very little research has focused on the impact of the training procedure on the behaviour and welfare of the individuals in their social context and daily life, after the training session took place (Spiezio et al., 2015). Similar studies are important to verify whether possible acute stress experienced from the animal during the training procedure might result in chronic stress, impacting on overall animal welfare. Although some authors (Mellen & MacPhee 2001; Spiezio et al. 2015) suggest an enriching effect of training on zoo animals, the literature is characterized by a lack of empirical evidence on costs and benefits (Melfi, 2009). Further studies are necessary to improve our knowledge of training and provide reliable cues for assessing the value and effectiveness of the PRT technique (Schapiro et al., 2003).

Several non-human primate species, such as apes (*e.g.*, bonobos [*Pan paniscus*]: Bell & Kahan, 2001; Clyde et al., 2001; Behringer et al., 2014. Sumatran orangutans [*Pongo abelii*]: Vandevoort et al., 1993; Behringer et al., 2014. Bornean orangutans [*Pongo pygmaeus*]: Moore & Suedmeyer, 1997. Chimpanzees [*Pan troglodytes*]: Lambeth et al., 2005; 2006; Perlman et al., 2004; Pomerantz & Terkel, 2009; Russell et al., 2006. gorillas [*Gorilla gorilla*]: Bond, 1991; Kuhar et al., 2005), rhesus macaques (*Macaca mulatta*) (*e.g.*, Coleman et al., 2008; Reinhardt, 2003) and callithricids (common marmoset [*Callithrix jacchus*]: Anzerberger & Grossweiler, 1993. Squirrel monkeys [*Saimiri boliviensis*]: Gillis et al., 2012. Golden-lion tamarin [*Leontopithecus rosalia*], Geoffroy's marmoset [*Callithrix geoffroyi*] and emperor tamarin [*Saguinus imperator*]: Smith et al., 2004), have been trained in the past few decades. However, little information has been published about training ring-tailed lemurs (*Lemur catta*), and strepsirrhines in general, although they are very common in both zoos and laboratories.

Previous research underlined that PRT can be enriching for captive animals (Bloomsmith, 1992; Melfi, 2013; Westlund, 2014) as it helps them to perform a wider array of marginal behaviours such as being separated from other group members for a short time or entering a specific area of the enclosure (Laule, 1992; Laule & Desmond, 1992).

The ring-tailed lemurs are strepsirrhines endemic to Madagascar; they are highly social primates and live in multi-male/multi-female social groups of about 11–35 individuals (Sussman, 1991). Ring-tailed lemur social interactions are very frequent, especially during feeding and sunbathing. As well as other primates, lemurs interact with each other through physical, visual and olfactory communication (Ganzhorn & Kappeler, 1993). Moreover, males and females rest and sleep in contact (Sauther, 1991; Sauther et al., 1999; Shire, 2012).

Given that the ring-tailed lemur is a social species, isolation of subjects could be stressful for both the individual separated from the group and for those left behind (Schapiro et al., 2003; Spiezio et al., 2015; Westlund, 2015). On the other hand, being able to reward single animals in isolation from conspecifics may be a valuable tool for their husbandry and well-being, allowing the provision of medicines to subordinate subjects, preventing aggression from conspecifics. Isolation training might be helpful to prevent and avoid the long-term separation of sick or injured subjects from their social group and to manage social tensions. More empirical findings are needed to assess the efficacy of positive reinforcement training in enhancing captive animal husbandry and research procedures (Schapiro et al., 2003).

This study investigated the effect of temporary isolation PRT on the behaviour and welfare of a captive group of ring-tailed lemurs hosted at Parco Natura Viva, Italy.

Animal behaviour has been found to be a key indicator of their health and well-being. Measuring behaviour is therefore helpful to assess animal welfare and is commonly used in the effectiveness evaluation of environmental enrichment programmes (Dawkins, 2004; Hill & Broom, 2009). Indeed, scientific studies (e.g., behavioural observation of time-budgets) and training programmes are required, before concluding that any measure will increase the complexity of an animal environment, might enhance its welfare (Weed & Raber, 2005; Baumans et al., 2011). As PRT has been found to be enriching for zoo animals, the current study focused on the effect of the isolation training programme on animal behaviour and welfare, using the same procedure involved in the effectiveness evaluation of environmental enrichment programmes.

The training programme focused on separation of individuals from the social group, and keeping them calm while isolated and make them retrieve rewards given by the trainer. First, we aimed at evaluating the effect of temporary social isolation obtained through the PRT programme on the welfare of the lemurs outside the training sessions (Bloomsmith, 1992; Schapiro et al., 2003). In order to achieve this aim, we compared the behaviour of the lemurs before the beginning of the training programme (baseline period) and during the training programme, after each isolation training session, when all lemurs were regrouped in their social context. In addition, to verify the number of sessions required from the lemur to enter the training area calmly and retrieve rewards, we collected behavioural data within each isolation training session.

If the social isolation through PRT had a positive effect on the welfare of lemurs and could be considered a kind of enrichment, we would predict a qualitative and quantitative increase in the performance of species-specific natural behaviour (Hosey et al., 2013). On the other hand, if the training programme was to impact negatively on the welfare of the lemurs, modifications in the lemur behavioural repertoire would be expected; in particular, abnormal and stress-related behaviour as well as aggressive behaviour would be displayed more than before the beginning of the training programme.

2. Materials and Methods

2.1 Subjects and Housing

Eleven adult ring-tailed lemurs were involved in this study (5 males and 6 females) and housed at Parco Natura Viva, Italy (Table 1). The enclosure comprised an indoor (80 m^2) and outdoor (1083 m^2) area. A small compartment (6 m^2) linked to both the indoor and outdoor areas of the enclosure through guillotine doors was used as the training area. The compartment contained a wooden shelf and a platform. Moreover, there was a small wire mesh hallway, not accessible to the lemurs, from which the trainer could handle the guillotine doors' opening and closing. During the training, the trainer was in the hallway and provided rewards to the lemurs throwing them on the wooden platform, without being in contact with the animals (Figure 1). The study lemurs were familiar with other basic kind of PRT; indeed, they were used to being moved from the indoor to the outdoor area of the enclosure and vice-versa. Non-invasive techniques were employed in the study and all lemurs were free to decide whether to enter the training area and take part in the training sessions or not. The research procedure was in accordance with the EU Directive 2010/63/EU and the Italian legislative decree 26/2014 for animal research. The research was approved by Parco Natura Viva committee and by the local authority.

2.2 Procedure

The study consisted of two different periods, a baseline period and an training period. The baseline period was a period without the training sessions, whereas the training period was in parallel with the isolation training sessions. In particular, the baseline period was run previously to the beginning of the habituation to the training area. The habituation was necessary to get the lemurs used to a new area and lasted for three weeks, before the beginning of the isolation training programme. During the habituation, the guillotine doors of the training area were always open and all lemurs could enter and retrieve rewards. During the training period, lemurs were trained to be isolated one by one and retrieve rewards given by the trainer on a specific platform in the training area, without visual and physical contact with the other members of the colony.

Before beginning the isolation training sessions, all lemurs were closed inside the indoor area of the enclosure. Then, each lemur was required to enter the training area and retrieve rewards given by the trainer in isolation from conspecifics; the reward was given to the lemurs after the guillotine doors were closed and they were calmly sitting on a platform fixed to the mesh of the training area. At the end of each session, the individual lemur went out to the outdoor area of the enclosure, to join the rest of the lemurs who has already done the isolation training session. The reward was a piece of primate jelly consisting of vegetables, fruits, nuts and honey (Viten®, Udine, Italy), pleasant for the lemurs and with low fat and sugar content. Each reward was a cube with a side length of approximately 1.5 cm. During the training, lemurs could enter the training area in a random order.

The indoor area of the enclosure housing all lemurs before they entered the training area was a transparent glasshouse. Therefore, lemurs could always see their conspecifics that finished the training session and were in the outdoor area of the enclosure and were able to make informed decisions on when to enter the training area, basing on the high-rank conspecifics' behaviour and position. Lemurs were free to choose whether to participate in the isolation training sessions or not. If they decided not to enter the training area, they remained in the indoor area and were released in the outdoor enclosure at the end of all training sessions. In particular, if no lemurs entered the training area after five minutes, the training sessions fnished and the subjects that remained in the indoor area were considered not willing to participate in the training session.

To prevent and minimize the stress of the lemurs, the duration of each habituation and training session varied according to the subject emotional state but never exceeded 3 minutes per lemur (Spiezio et al., 2015).

In both the baseline and the training periods a continuous focal animal sampling was used (Altmann, 1974) to collect data on the behaviour of the lemurs in the social context. In the baseline period, twenty-two 10-min sessions per lemur were run . In particular, for each subject, two sessions per day were carried out, one in the morning and one in the afternoon, over a two week period. Given that the training programme was made of nine isolation training sessions necessary to have 100% of lemurs achieved the training goal, in the training period eighteen 10-min sessions per lemur were carried out. In particular, one session started in the morning, immediately after the isolation training session when all lemurs were again in their social context, one in the afternoon. In both the baseline and training periods, data on individual (sleeping, attentive behaviour, exploration, foraging, locomotion, maintenance, scent-marking, self-grooming and sunning) and social (agonistic behaviour, grooming, playing, social contact and social sleeping) behaviours were collected (Table 2). We also collected the time lemurs spent out of sight. Several wild animal species tend to hide in order to escape negative stimuli or dangerous situations. Out of sight behaviour is particularly relevant in captivity: indeed, captive animals can sometimes try to hide or escape in the presence of stressors, such as the presence of visitors. Moreover, being out of sight might be informative of the animal state or chronical stress and could therefore be relevant in the evaluation of zoo animal welfare (Carlstead et al., 1993; Sellinger & Ha, 2005; Davey, 2006; Morgan & Tromborg, 2007; Hosey, Melfi & Pankhurst, 2013).

The training programme period ceased when all lemurs entered the training area and retrieved the reward from the trainer for an arbitrary number of four consecutive sessions. As not all lemurs were calm enough to retrieve the reward in the first five sessions but started to do that consecutively from the sixth session, a total of nine sessions were completed. One training session per day was done and each session was carried out in the early morning, always at the same time. Within the training sessions, information on the responses and behaviours of the lemurs inside the training area were recorded. For each lemur and for each training session, data on the frequency of participation (voluntary entering the training area and being separated from conspecifics) and retrieving of the reward were recorded; furthermore, data on the latency to enter the training area and to retrieve the reward as well as on the duration of stress-related behaviours (self-scratching and licking, defecation, urination and stereotypes, particularly head-rotation) were collected. All training sessions were videotaped. Data were collected through the observations of the video-tapes, using a continuous focal animal sampling method (Altmann, 1974). The data from the training sessions were analysed in order to assess whether the lemurs were fully prepared to remain isolated from the rest of the group and to voluntarily participate in husbandry, veterinary care and research, outside the social context.

2.3 Data Analysis

Kolmogorov-Smirnov test (K-S) revealed that not all data were normally distributed. Therefore, data were analysed using non-parametric statistical tests to compare the behaviour of the lemurs between the baseline and training periods. Data analyses were performed using the statistical software *StatView 5.0* by SAS Institute Inc. Per period, the percentage duration of each behavioural category in relation to the whole total time of observation (Baseline: 220 minutes, Training: 180 minutes) was calculated and considered in all the analyses. Wilcoxon test was used to compare behavioural data between the baseline and the training periods. Furthermore, the behaviours and responses of the lemurs as training progressed were evaluated using Spearman correlations. In particular, the mean latency per session over all subjects to enter the training area and to retrieve the rewards was correlated with the number of sessions; in addition, the mean duration per session over all subjects of stress-related behaviours was correlated with the number of sessions. Finally, Mann-Whitney test was used to assess whether the sex of the subjects affect their response within the training sessions. Percentage mean ± SD durations in seconds of individual and social behaviours are reported in brackets alongside the result section.

3. Results

3.1 Effect of the training programme on the lemur behaviour: baseline period vs training period

Species-specific individual and social behaviours were displayed by the lemurs and no abnormal behaviours were reported in both the baseline and training periods. Regarding the effect of the training on the lemurs, the training programme seemed to partially affect the behaviour of the study subjects (Figure 2). In particular, inactive behaviours (individual and social sleeping) were performed significantly less in the baseline (5.40 ± 3.90) than in the training period (20.80 ± 10.93) (Wilcoxon test: $z = -2.756$, $p = 0.006$, N = 11; Figure 2, 3). No significant differences were found for active behaviour (baseline: 79.14 ± 7.97; training period: 74.84 ± 11.70) (Wilcoxon test: $z = -0.711$, $p = 0.478$, N = 11; Figure 3). In addition, out of sight was performed significantly more in the baseline (15.46 ± 5.20) than in the training period (4.36 ± 2.89) . (Wilcoxon test: $z = -2.934$, $p = 0.003$, N = 11; Figure 2, 3).

No significant difference in overall individual behaviour (baseline: 72.02 ± 6.49; training period: 63.84 ± 12.80) between the two periods was found (Wilcoxon test: $z = -1.600$, $p = 0.110$, N = 11) (Figure 4). In particular, within individual behaviour, exploration, locomotion and sunning were performed significantly less in the training period than in the baseline period (Table 3, Figure 2).

Overall social behaviours were performed significantly less in the baseline (12.52 ± 5.14) than in the training period (31.80 ± 12.34) (Wilcoxon test: $z = -2.845$, $p = 0.004$, N = 11) (Figure 4).. In particular, within social behaviours, agonistic behaviours were displayed significantly less in the training than in the baseline period. On the contrary, social contact and other affiliative behaviours (grooming and play) were displayed more in the training than in the baseline period. A significant difference between the two periods was found for social contact but not for other affiliative behaviour (Table 3, Figure 2).

Regarding inactive behaviour, both social and individual sleeping were performed significantly less in the baseline (social sleeping: was 3.44 ± 2.61; individual sleeping: 1.97 ± 2.17) than in the training period (social sleeping: 12.79 ± 9.45; individual sleeping: 8.01 ± 5.26) (Wilcoxon test: z = - 2.312, p = 0.021 and z = - 2.845, p = 0.004, N = 11, respectively).

3.2 Lemur response within the training session

Nine sessions were required to the lemurs in order to retrieve the reward for four sessions consecutively. Within the training sessions, each lemur reacted differently to the training programme. Seven out of 11 lemurs took part in all training sessions, whereas five of the 11 subjects always took the rewards during the training. However, all of the lemurs participated in a minimum of 67% of the training sessions. In the context of stress-related behaviours, six of the 11 subjects displayed stress-related behaviours during training (Table 1).

No significant correlation was found between the latency to enter the training area and the nine sessions (Spearman correlation: *rho* = -0.583; p = 0.099, N = 9; Figure 5). The same result was obtained when the duration of stress behaviours was considered (Spearman correlation: *rho* = 0.018; p = 0.958, N = 9). On the other hand, a negative correlation between the latency to retrieve the reward and the nine sessions was found (Spearman correlation: *rho* =-0.667, p = 0.049, N = 9; Figure 5).

The Mann-Whitney test did not reveal any differences between females and males in the latency to enter the training area (1454.17 ± 1509.36 and 520.20 ± 324.14 respectively) and in the latency to retrieve the reward (931.83 ± 883.36 and 848.80 ± 683.61 respectively) (latency to enter: U = 10; p = -0.812; latency to retrieve the reward: U = 15; p = 0.091). Smilarly, no significant difference between females and males was found in the performance of stress-related behaviour (22.83 ± 42.19 and 80.60 ± 79.51 respectively) (U = 10; p = -0.812).

4. Discussion

During both the baseline and the training period, when the lemurs were living in their social context and every day environment, species-specific individual (e.g., locomotion, attentive behaviour, feeding and sunbathing) and social behaviours (e.g., grooming, social contact) were reported. No abnormal and stress-related behaviours were recorded. In particular among individual behaviours, activities such as feeding and sunbathing are very typical of this species (Ganzhorn & Kappeler, 1993). In addition, lemurs are highly social primates and grooming, resting and sleeping in contact are examples of social interactions (Sussman, 1991, Sauther, 1991, Sauther et al., 1999). As animal welfare is the state of an animal as regards its attempts to cope with its environment (Broom, 1996) and thus the ability to respond to a range of stimulation, findings of this study suggest that the study colony was in a good state of welfare, since a variety of normal and specific behaviours are performed (Hill & Broom, 2009; Hosey et al., 2013).

However, differences were found between the two periods. In particular, we observed a significant increase in inactive behaviours, both social and individual sleeping, in the training period compared to the baseline period; this finding is not consistent with other research on PRT in non-human primates. Previous studies reported an increase in activity levels as a consequence of animal training (Bloomsmith, 1992; Desmond et al., 1987). A possible explanation could be that lemurs need to rest after the training requirements and mental stimulation. Importantly, inactive behaviour is never performed in excess by the study lemurs (approximately 5% of the total time in the baseline and 21% in the training period) as wild ring-tailed lemurs have been found to spend more than 50% of the time in inactive behaviour (Ellwanger & Gould, 2011; Jolly, 1996; Keith-Lucas et al., 1999). Therefore, as the study subjects behave similarly to their wild counterpart in both the baseline and training period, the increased inactivity seems not to highlight a negative effect of the isolation training programme. On the other hand, during the training period, lemurs were out of sight less often than during the baseline period. Captive animals may sometimes need to hide from stressors or negative stimuli, such as the presence of humans (e.g., zoo visitors). The out of sight behaviour might therefore be informative on the state and chronical stress of the subjects (Carlstead et al., 1993; Sellinger & Ha, 2005; Davey, 2006; Morgan & Tromborg, 2007; Hosey, Melfi & Pankhurst, 2013). Thus, the decrease in the performance of out of sight behaviour reported in the study ring-tailed lemurs suggests that they spent more time facing the presence of the human observer and caregivers. According to previous studies on chimpanzees (Baker, 2004), the positive human-animal interactions involved in PRT have been found to reduce inter-specific aggression outside the training context. These findings support the hypothesis that PRT could be valuable for improving the coexistence between animals and humans (Bayne et al., 1993; Bloomsmith et al., 1997; Hosey & Melfi, 2012, Westlund, 2015). In addition, ring-tailed lemurs were often out of sight when sleeping on branches and foliage at the top of the trees, in the back of the enclosure. Therefore, it is possible that in the baseline period, when lemurs were in the out of sight condition, they were simply sleeping in trees. The increase of inactive behaviour in the training period might be due to the fact that ring-tailed lemurs, coping better with the presence of humans, spent more time sleeping on lower branches or other places where they were more visible and closer to humans.

Within the individual behaviours, exploration, locomotion and sunning were performed significantly less in the training than in the baseline period. These findings may be linked to the increase in inactive behaviour in the training period. Nevertheless, since locomotion has been previously associated to stressful situations (Archer, 1973; Ramos & Mòrmede, 1998), the significant decrease in locomotion during the training period might indicate an enhanced welfare of the study lemurs. We have previously reported similar findings as an outcome of work on the effect of an isolation PRT programme in vervet monkeys (*Chlorocebus aethiops*) (Spiezio et al., 2015).

On the contrary, ring-tailed lemurs performed significantly more social behaviour during the training than the baseline period. In particular, a significant increase in social contact as well as a decrease in agonistic behaviour in the training period was found. These findings suggest that training may positively impact animal welfare by improving social relationships and reducing aggressive behaviour. The increase of affiliative behaviours, specifically social contact, could be presumably due to the need of the lemurs to re-establish social relationships and intra-group alliances after the isolation period (Moscovice et al., 2015; Reite & Capitanio, 2012). These findings support our current knowledge of the effect of training on animal social environments (Bloomsmith, 1992; Desmond et al., 1987; Laule, 1993; Manciocco et al., 2009; Spiezio et al., 2015), providing additional evidence that this practice could be beneficial for the husbandry management of captive primates, but also the overall well-being and social life of these animals. This study highlights an overall positive effect of the isolation training programme on the lemur behaviour and welfare outside the training sessions.

Regarding the behavioural responses of lemurs during the training session (within the training area), each subject responded differently to the training condition; as a result of this, individual variability might be hypothesized. As previously reported (Coleman et al., 2008; Wergård, 2015; Spiezio et al., 2015), it is possible that the temperament of the animals is relevant for training efficacy in terms of required learning time, initial trainability and time spent to overcome the fear of the trainer. However, according to our results, within nine sessions, all the study lemurs achieved the training goal to participate voluntarily in training sessions and to remain isolated from the rest of the group by being motivated trough a positive reward. Thus, husbandry, veterinary care, research, and consequently the welfare of lemurs could be improved with this procedure as observed for laboratory primates species (see Prescott & Buchanan-Smith, 2003; Prescott et al., 2005; Westlund, 2015)

Stress-related behaviours were performed only in the training area, during the training session, in which lemurs were isolated from conspecifics, suggesting that being isolated from the rest of the group could be a stressful event for a social species (Schapiro et al., 2003; Spiezio et al., 2015; Westlund, 2015). However, the acute stress of the training procedure seems not to impact on the welfare of the lemurs when they went back to their social context and daily environment. Furthermore, the absence of any correlation between the training sessions and both the latency to enter the training area and the duration of stress-related behaviours suggest that habituation to the training area and procedure is not easily manageable by captive lemurs. On the other hand, a significant negative correlation between the sessions and the latency to retrieve the reward was found, suggesting that trust between the trainer and ring-tailed lemurs is reasonably easy to build.

However, at the beginning of the training the progress of the learning process might be slow, requiring a certain number of sessions before animals start to learn quickly (Westlund, 2015).

No significant differences in the response of the lemur within the training sessions between females and males were found. This finding is in agreement with previous studies on common marmosets (*Callithrix jacchus*), reporting no sex differences in the number of sessions required to accomplish target and urine training correctly (McKinley, Buchanan-Smith, Barrett & Morris, 2003). However, previous studies on chimpanzees reported that females required fewer training sessions than males to accomplish the training goal, suggesting possible effects of sex on PRT programmes (Schapiro et al., 2003). Further studies should involve a greater sample of females and males to assess the influence of sex on the response of the lemurs to the training programme.

In conclusion, when used to isolate individuals from their social context, the PRT technique might be stressful for group-living primate species, including lemurs. However, our results highlight that this technique might improve the everyday social life of captive primates, thus representing a good management tool. Therefore, this study provides further evidence that PRT is useful for enhancing animal husbandry and welfare in captivity, not only during training sessions but also in their social environment. Voluntary isolation of animals obtained using PRT offers the opportunity of animal models and regular health check of the subjects, representing a valid tool to enhance the animal daily life as well as the quality of scientific data (Laule, Bloomsmith & Schapiro, 2003; Scott et al., 2003). Furthermore, we believe this work contributes to filling the gap in the literature regarding empirical data related to the impact of training on animal well-being outside the period of training sessions (Melfi, 2013). Our study also highlights the importance of research and practice aimed at improving the husbandry standards for captive animals by designing adequate species-specific training programmes (Schapiro et al., 2003).

AUTHOR DECLARATION

We wish to draw the attention of the Editor to the following facts which may be considered as potential conflicts of interest. Caterina Spiezio is employed by Parco Natura Viva as head of the Research and Conservation Department. The category of potential conflict of interest is “Employment”. Barbara Regaiolli is employed by Parco Natura Viva as researcher in the Research and Conservation Department. The category of potential conflict of interest is

"Employment". All these authors have disclosed those interests fully to Elsevier and they have in place an approved plan for managing any potential conflicts arising from the involvement.
Stefano Vaglio and Consuelo Scala have no conflict of interest.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved animals has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from. The email address is S.Vaglio@wlv.ac.uk

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Acknowledgements

We would like to thank Parco Natura Viva – Garda Zoological Park Director, Cesare Avesani Zaborra, and General Curator, Camillo Sandri, for allowing this study. In addition, special thanks go to Paola Maria Valsecchi (Parma University) for her support and advice during the study. We thank the anonymous reviewers for the careful reading of our manuscript and the useful comments and suggestions.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Figure captions

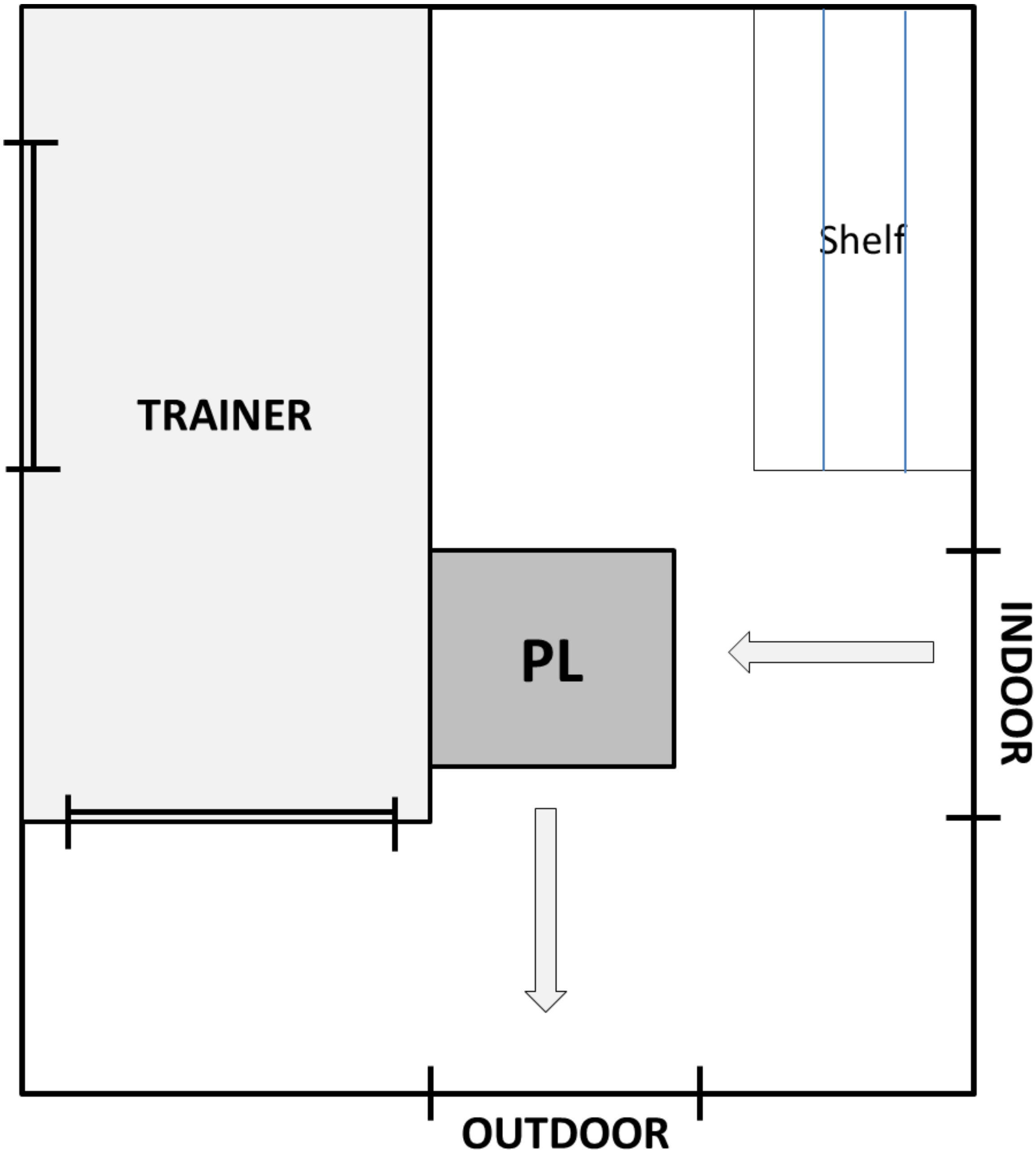
Figure 1. Training Area. Schematic representation of the small compartment in which the isolation training sessions were run (training area). The compartment was linked to both the indoor (INDOOR) and outdoor (OUTDOOR) areas of the enclosure through guillotine doors. Each lemur entering the compartment coming from the indoor area had to retrieve rewards on the platform (PL) and was then released in the outdoor area of the enclosure. The trainer provided the lemurs with rewards from a wire mesh hallway (TRAINER) and no human-animal contact was allowed. The arrows indicate the expected route of the lemur from the beginning to the end of the training session..

Figure 2. Behaviours performed by the study ring-tailed lemurs. The pie charts report the mean duration (%) of different behaviours performed by the lemurs in the baseline (1) and in the training period (2).

Figure 3. Comparison of activity, inactivity and out of sight behaviour between the baseline and the training period. Mean duration (%) in seconds of the behaviours performed by the lemurs are reported; error bars represent +/- 1 standard deviation. * $p < 0.05$.

Figure 4. Comparison of individual and social behaviour between the baseline and the training period. Mean duration (%) in seconds of the behaviours performed by the lemurs are reported; error bars represent +/- 1 standard deviation. * $p < 0.05$.

Figure 5. Correlations between the mean latency over all lemurs to enter the training area (light grey line) and to retrieve the reward (dark grey line) and the nine PRT sessions. Error bars represent +/- 1 standard deviation.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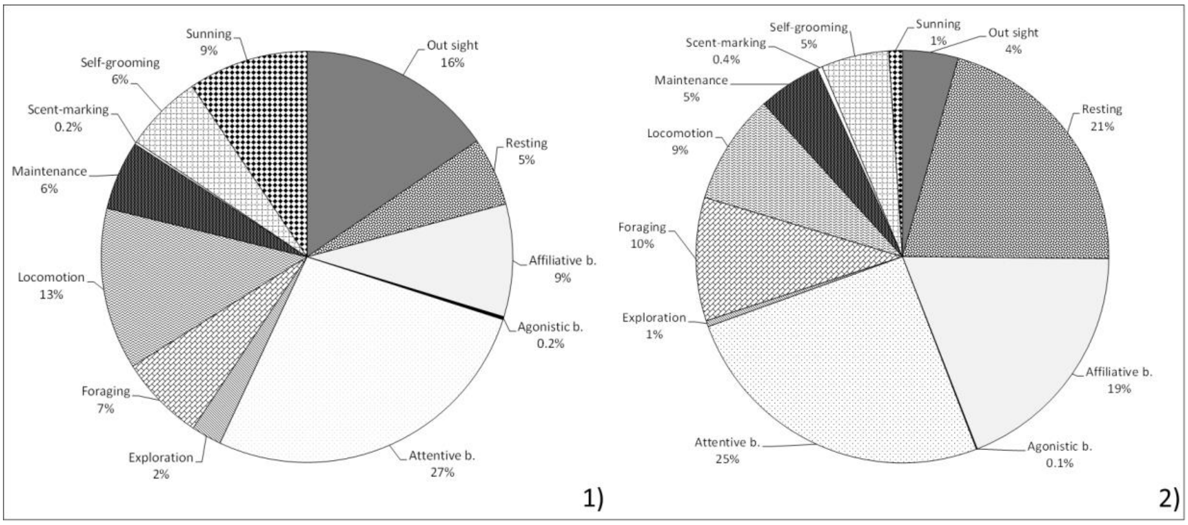
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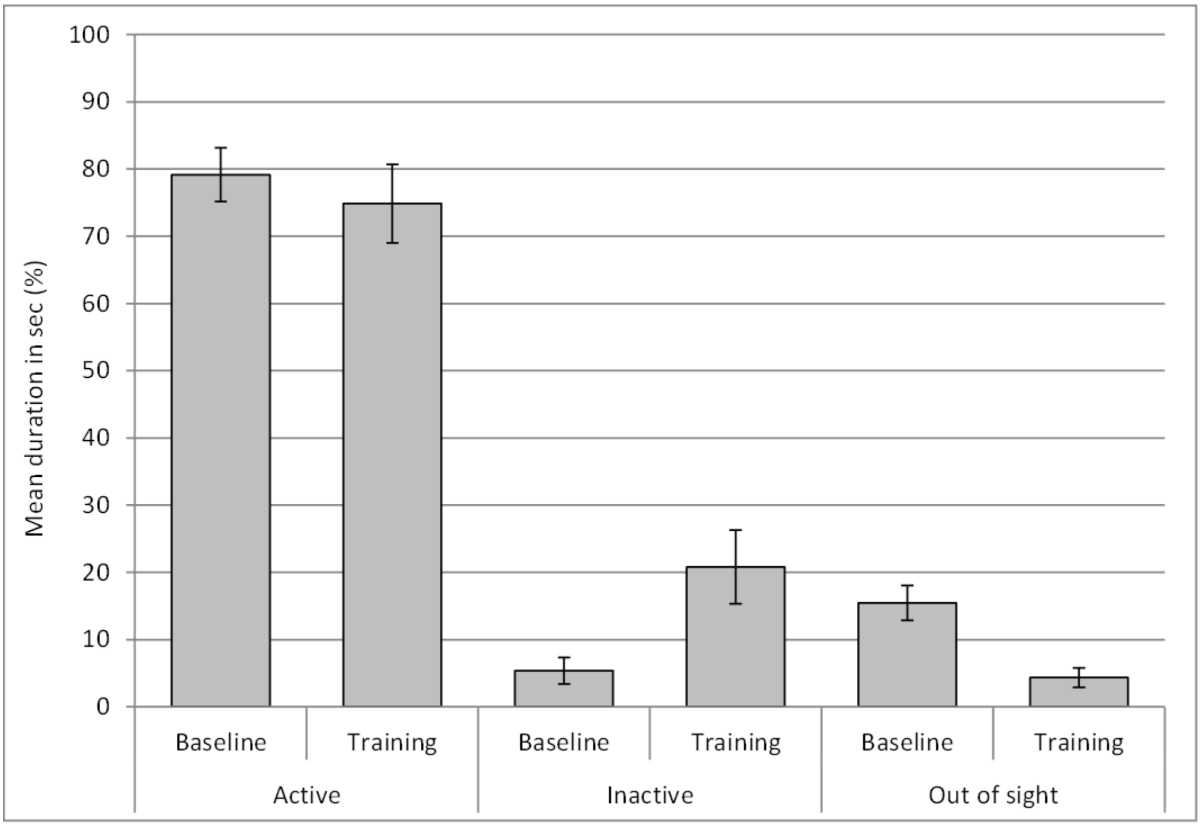
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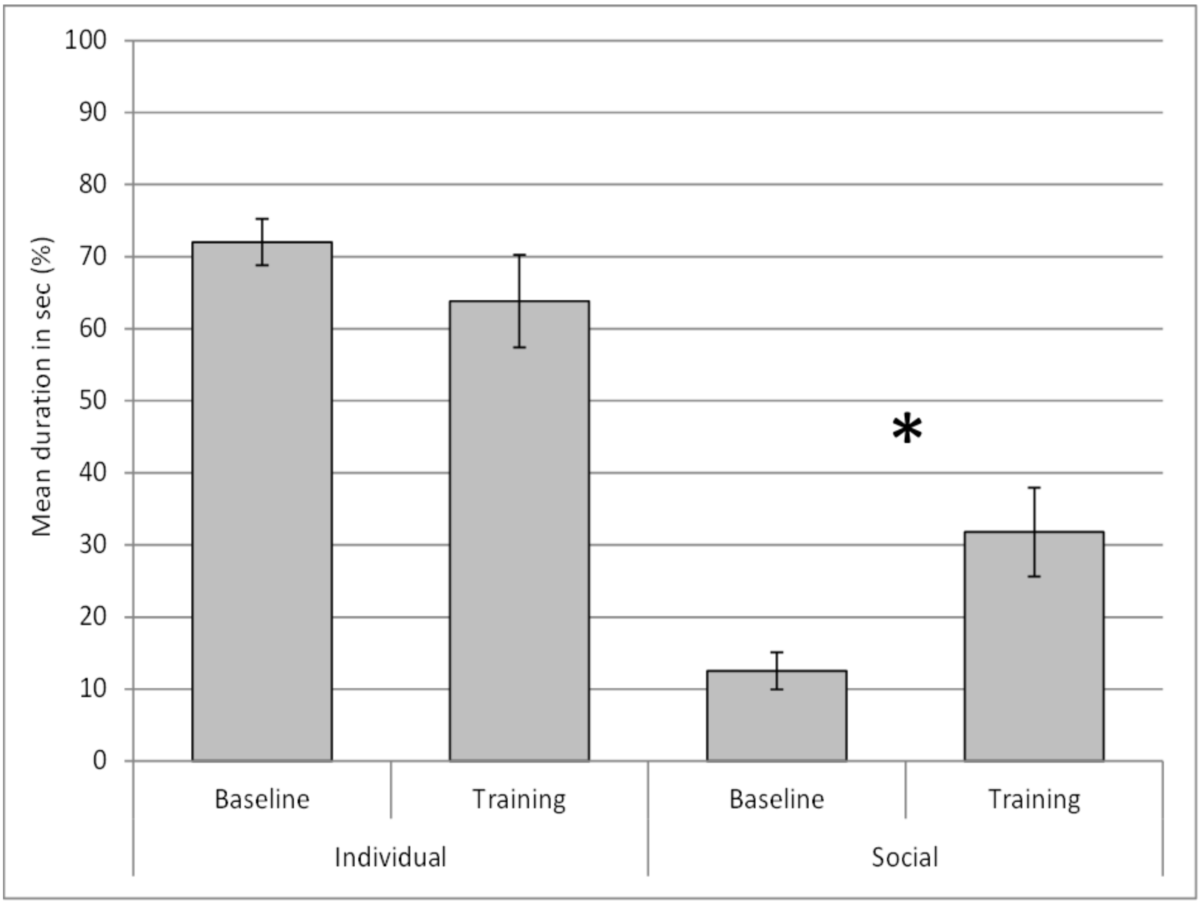
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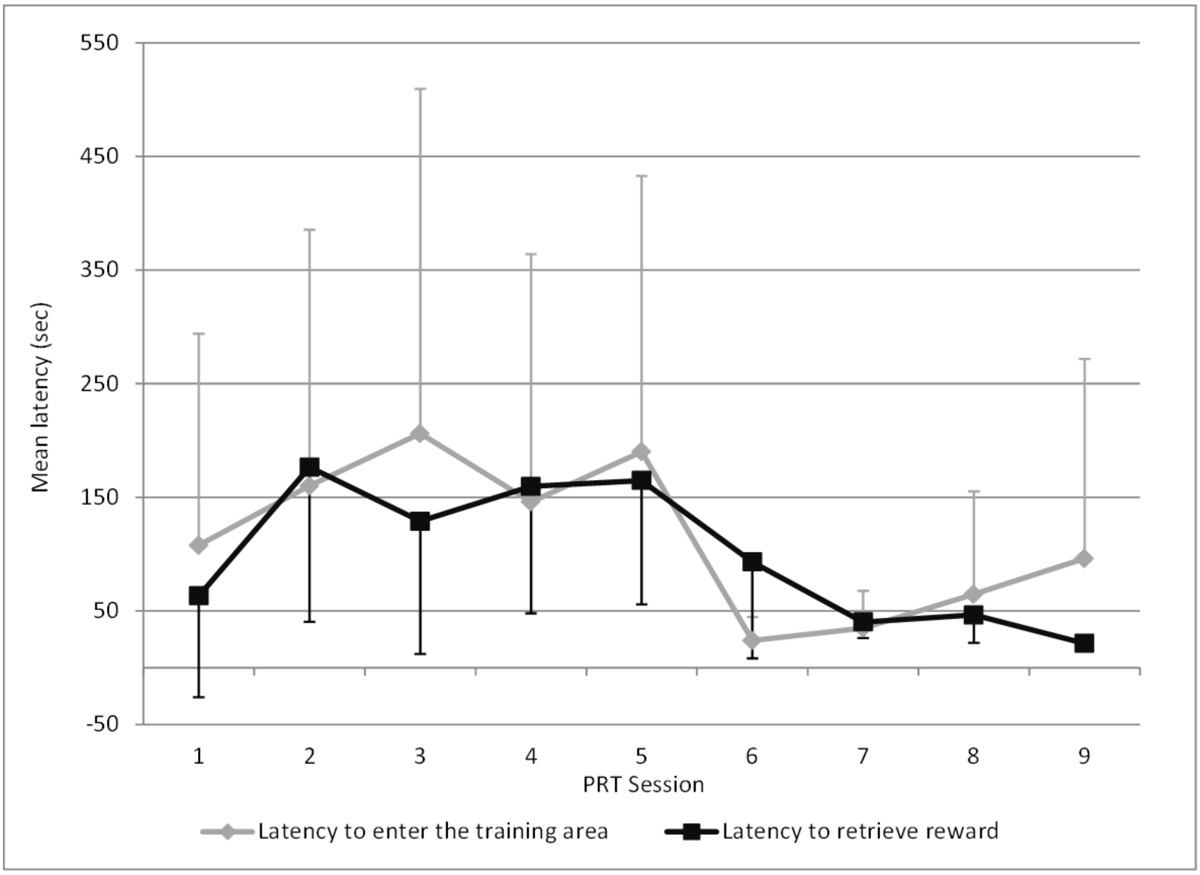
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PRT Session
Latency to enter the training area
Latency to retrieve reward

Table 1. Subject of the study and response within the training sessions. The table reports the sex (F = female; M = male) and the age (years at the time of data collection) as well as the total and % (in brackets) number of sessions in which each subject participated (Training), the total and % (in brackets) number of sessions in which each subject retrieved the reward from the experimenter (Reward) and the mean duration ± SD (in seconds) of stress behaviour (Stress) across all training sessions.

Subjects	**Sex**	**Age**	**Training**	**Reward**	**Stress (sec)**
Bah	F	6	8 (89%)	8 (89%)	0 ± 0
Sally	F	6	9 (100%)	9 (100%)	0 ± 0
Nad	F	4	9 (100%)	9 (100%)	2.56 ± 6.31
Nea	F	7	6 (67%)	6 (67%)	1.00 ± 2.65
Neretta	F	13	7 (78%)	6 (67%)	15.30 ± 32.84
Oscar	F	5	9 (100%)	9 (100%)	0 ± 0
Bicolor	M	3	9 (100%)	8 (89%)	19.40 ± 20.70
Zeno	M	3	8 (89%)	8 (89%)	0 ± 0
Indi	M	3	9 (100%)	9 (100%)	0 ± 0
Pierrot	M	3	9 (100%)	7 (78%)	15.30 ± 29.32
Step	M	3	9 (100%)	9 (100%)	10.00 ± 30.00

Table 2. Ethogram used in the baseline and training periods.

Behavioural categories	Description
Inactive behaviours	
Sleeping	Sleeping, being inactive with closed eyes.
Social sleeping	Sleeping in contact with one or more individuals.
Individual behaviours	
Attentive behaviour	Looking around while sitting, laying or standing.
Exploration	Sniffing, licking or interacting with an object, a surface or the environment.
Foraging	Searching for food in the grass or in hay/straw mounds, far from the feeding points (bowls).
Locomotion	Walking or running, moving on branches or other substrates.
Maintenance	Eating from the food bowl, drinking, licking mineral salt blocks, defecating, urinating.
Scent-marking	Marking the environment with brachial, ante-brachial or genital glands. Urinating with the tail held up (urine marking).
Self-grooming	Self-licking, self-cleaning of the body.
Sunning	Sitting with open arms and the ventral part of the body directed toward the sun.
Social Behaviours	
Agonistic behaviours	Supplanting, chasing, threatening, cuffing, pushing away, hitting.
Other affiliative behaviours	Grooming and playing.
Social contact	Being in contact with one or more conspecifics.
Out of sight	
Not visible	The observer can see the lemur but not the behaviour being performed.
Out of sight	Being out of sight, hiding in tree or areas of the enclosure which are distant from the visitors and the human observer. Both the lemur and the behaviour performed are not visible.

Table 3. Individual and social behaviours in the baseline and training period. The table reports the % mean duration in seconds (M) and the standard deviation (SD) of each behavioural category in both the baseline and the training period, the *Z*-value and the *P*-value from the Wilcoxon test for the comparison between the two periods. Asterisks indicate behavioural categories for which a significant difference between the two periods was found (Wilcoxon test: $p < 0.05$).

		Baseline (N = 11)	**Training (N = 11)**	***z-value***	***p*-value**
Individual	**Attentive b.**	27.01 ± 2.64	25.33 ± 9.07	-0.445	0.660
	Exploration	2.38 ± 1.17	0.48 ± 0.29	-2.934	0.004
	Foraging	6.74 ± 3.00	9.66 ± 3.62	-1.778	0.075
	Locomotion	12.74 ± 3.61	8.65 ± 3.07	-2.756	0.006
	Maintenance	5.44 ± 2.68	4.95 ± 3.60	-0.711	0.477
	Scent-marking	0.20 ± 0.34	0.40 ± 0.90	-0.255	0.802
	Self-grooming	6.13 ± 1.77	5.30 ± 4.18	-0.800	0.424
	Sunning	9.42 ± 3.10	1.06 ± 0.84	-2.934	0.003
Social	**Agonistic b.**	0.23 ± 0.15	0.07 ± 0.07	-2.845	0.004
	Other affiliative	4.28 ± 2.80	4.85 ± 1.32	-0.800	0.424
	Social contact	4.58 ± 2.73	14.09 ± 6.00	-2.845	0.004